Carbohydrate Regulation

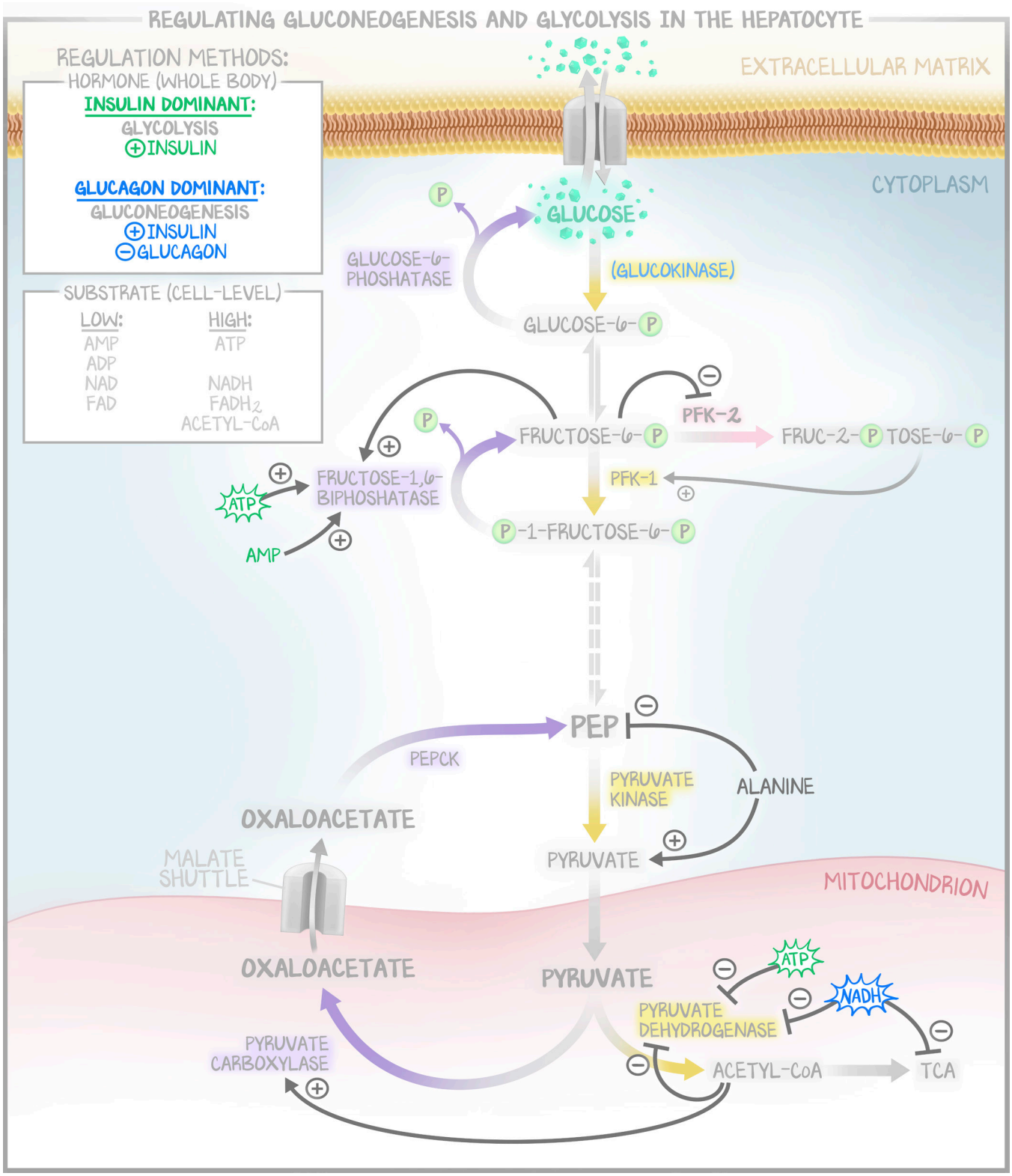

Figure 9.1: Regulating Glycolysis and Gluconeogenesis in the Hepatocyte
We show both gluconeogenesis and glycolysis regulation at the same time. Notice that glucagon doesn't fight insulin—each hormone either activates or fails to activate its own target.

Introduction

The regulation discussed here occurs **only in hepatocytes** because **only the liver** can switch between glycolysis and gluconeogenesis, between insulin and glucagon. Placing this lesson here helps to review the two pathways, gluconeogenesis and glycolysis. It helps to see them oppose one another, to see gene expression vs. phosphorylation, to lay them out. What we are leaving out is the integration of other systems—glycogen, fatty acids—which will come later. This lesson is supposed to be a summary of the key enzymes and their regulation as it all relates specifically to carbohydrates.

Carbohydrate metabolism has two levels of control: hormone and substrate. Just as a cruise ship has a destination port (New Orleans to St. Maarten), the liver has a destination (glucagon-dominant state or insulin-dominant state). Hormones set the destination. And just as everyone on that cruise ship will arrive at St. Maarten together, so too will the hepatocytes all be directed toward either glucagon or insulin. But the passengers' activities en route—what they do, when they do it, and with whom they do it—can vary wildly. Some may go to the casino and never leave the bar, while others may spend all their time on deck and never see a drink. It's this variability of activities that is a metaphor for subtrate-level control. Hormones set the destination, substrates determine what activities are being done while the ship is on its way.

Hormone-level control is whole-body. The pancreas uses hormones to create an insulin-dominant environment for the liver (which in this lesson means glycolysis), or a glucagon-dominant environment (which in this lesson means gluconeogenesis). The global perspective of insulin vs. glucagon is what the two introductory lessons were about. And then we learned that there was certainly more than just glycolysis and gluconeogenesis. This lesson will focus specifically on how i**nsulin and glucagon exert their effects on the enzymes of glycolysis and gluconeogenesis**, the mechanisms by which they do it, not just that there is a "glucagon world" or an "insulin world" in general. Do realize that more happens to more enzymes than we let on, but these are the ones relevant for carbohydrate metabolism.

Substrate-level control is within hepatocytes. This might seem confusing, but its relevance is apparent when we discuss priority. It would be challenging to explain a situation where the pancreas was releasing insulin (stimulating glycolysis) but the local liver environment was opposing it. Instead, while the pancreas has determined that the liver's "destination" is insulin, so that the liver is moving in that direction, **substrate-level control** helps the liver decide **which insulin-dominant pathways to prioritize**.

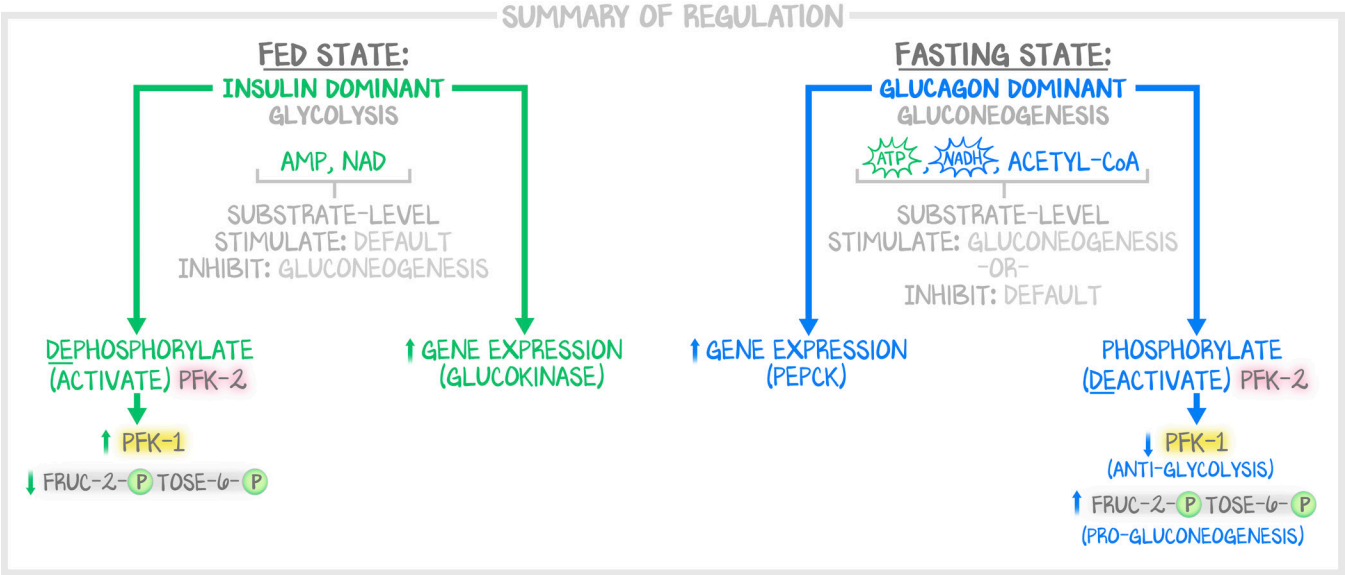

Figure 9.2: Summary of Regulation

A hepatocyte on its way to insulin will be performing the actions of burning and storage. The hepatocyte will burn glucose for ATP, store glucose as glycogen, and turn glucose into a stored form of energy called fatty acids. Storing glycogen and making fatty acids requires energy. The hepatocyte must first ensure that it has enough energy (prioritize glycolysis-TCA-ETC) before it attempts to use energy (ATP) to store energy for later (in the forms of fatty acids and glycogen). And since fatty acid and glycogen formation takes not only energy but also glucose, and glucose is needed to make energy, the hepatocyte should only use glucose and energy when both are abundant. This is a longwinded way of saying that **under insulin's influence, glycolysis is prioritized; and then, when ATP is abundant, still under insulin's influence, other storage pathways can be used**.

That is to say, **an abundance of low-energy compounds** (NAD, FAD, AMP) will stimulate/disinhibit the pathways that make high-energy compounds (glycolysis, PDH, TCA, ETC). Using the same logic, **an abundance of high-energy compounds** (NADH, $FADH_2$, ATP, acetyl-CoA) eschew pathways that make high-energy compounds and favor other pathways that can use these high-energy compounds for storage (glycogen storage, fatty acid synthesis).

Cytoplasm and Hormones Go Together

The hormones of the system—**insulin and glucagon**—do their battle in the cytoplasm . . . mostly. Their mechanism changes based on the enzyme. Rarely do hormones affect the same enzyme—insulin activating, glucagon deactivating. The example where they do is the regulation of PFK-2. Often it's the simultaneous **presence of one** activating its complement of enzymes AND the **absence of the other** failing to activate its complement that results in the final combined outcome.

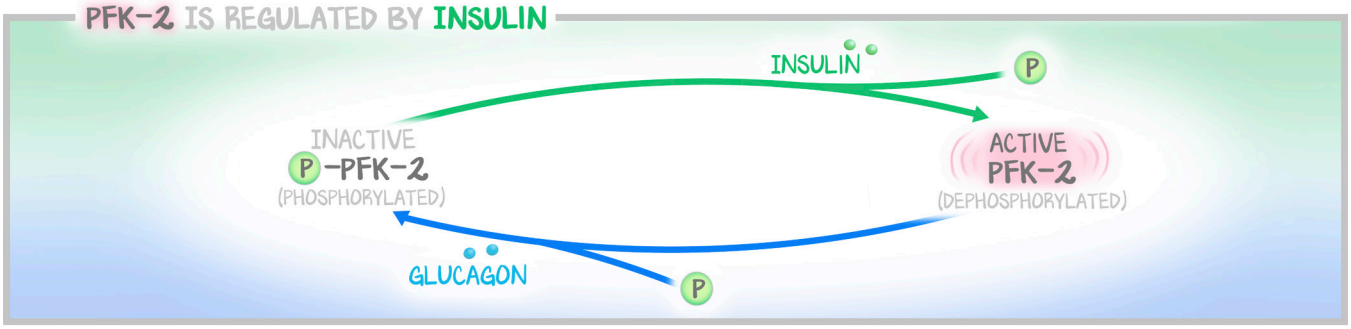

Figure 9.3: Regulation of PFK-2
Insulin dephosphorylates thereby activating PFK-2; glucagon phopshorylates thereby deactivating PFK-2.

Except for **insulin's stimulation** (via dephosphorylation) of the **mitochondrial enzyme pyruvate dehydrogenase**, carbohydrate regulation by hormones is limited to the cytoplasm. And insulin-stimulating PDH is more for fatty acid synthesis than it is for "carbohydrate regulation." Mitochondrial regulation is almost entirely substrate-level feedback. Cytoplasmic regulation is also regulated by substrate-level regulation, but it's in the cytoplasm where the hormones do the most work.

Enzymes in the cytoplasm may also be substrate-regulated, but mitochondrial enzymes are exclusively substrate-regulated (except PDH).

Insulin Tips the Scale Toward Glycolysis

Insulin **induces glucokinase**. Induction means **increased gene expression**. It's not as rapid as phosphorylation, but it's stronger and longer lasting. When insulin is produced, the genetic expression of hepatocytes shifts away from gluconeogenesis and toward glycolysis. Gluconeogenesis builds and releases glucose. Glucokinase traps glucose in the hepatocyte. They cannot both happen at the same time. The absence of insulin means there is less gene expression, and less production of glucokinase. The presence of insulin increases glucokinase expression.

Insulin also **dephosphorylates**, **thereby activating PFK-2**. We discussed this in detail in Metabolism #3: *Glycolysis* and again in Metabolism #8: *Gluconeogenesis*. Insulin dephosphorylates PFK-2, inducing a conformational change and **activating it**. PFK-2 makes fructose-2,6-BP. This substrate **stimulates PFK-1**. It is an indirect activation of PFK-1, and also an indirect inhibition of the opposing gluconeogenesis enzyme fructose-1,6-bisphosphatase. Less insulin means less PFK-1 activity.

Insulin **dephosphorylates**, **thereby activating PDH**. PDH uses the pyruvate made by glycolysis. Removing a downstream substrate ensures favorability of the downstream reaction AND ensures that pyruvate is converted to acetyl-CoA for either TCA-ETC or FA synthesis. Less insulin means less PDH activity. More insulin means more PDH activity (see why in Metabolism #13: *Lipid Synthesis*).

Glucagon Tips the Scale Toward Gluconeogenesis

Glucagon **induces PEPCK**. Induction means **increased gene expression**. It's not as rapid as phosphorylation, but it's stronger and longer lasting. When glucagon is produced, the genetic expression of hepatocytes shifts away from glycolysis and toward gluconeogenesis. PEP is common to both gluconeogenesis and glycolysis. Surplus PEP could theoretically go right back to pyruvate. But in the setting of other protein changes and substrate levels, having more PEP around just pushes the system toward glucose release.

Glucagon phosphorylates PFK-2. **Phosphorylation deactivates PFK-2.** With less PFK-2, there is less fructose-2,6-bisphosphate. With less fructose-2,6-bisphosphate, there is reduced stimulation of PFK-1 and a disinhibition of fructose-1,6-bisphosphatase.

Substrates and Their Enzymes

The enzyme of gluconeogenesis, fructose-1,6-bisphosphatase is stimulated by less fructose-2, 6-bisphophate. But fructose-1,6-bisphosphonate, the enzyme of gluconeogenesis, is also **stimulated by ATP** and **inhibited by AMP**. The very presence of ATP shifts the hepatocyte away from more glycolysis. This can also be seen as "toward glycogen storage." In the mitochondria, substrate levels are more elevated.

Pyruvate dehydrogenase is **inhibited by the products of the reaction**. It also keeps in line with the high-energy-state substrates turning off glycolysis-TCA-ETC. **NADH** inhibits PDH. **Acetyl-CoA** inhibits PDH. And the downstream product of NADH within the ETC, **ATP**, inhibits PDH. The only thing that stimulates PDH is **calcium**, and that's in muscle cells, not the hepatocyte. There's no smooth muscle to contract in hepatocytes.

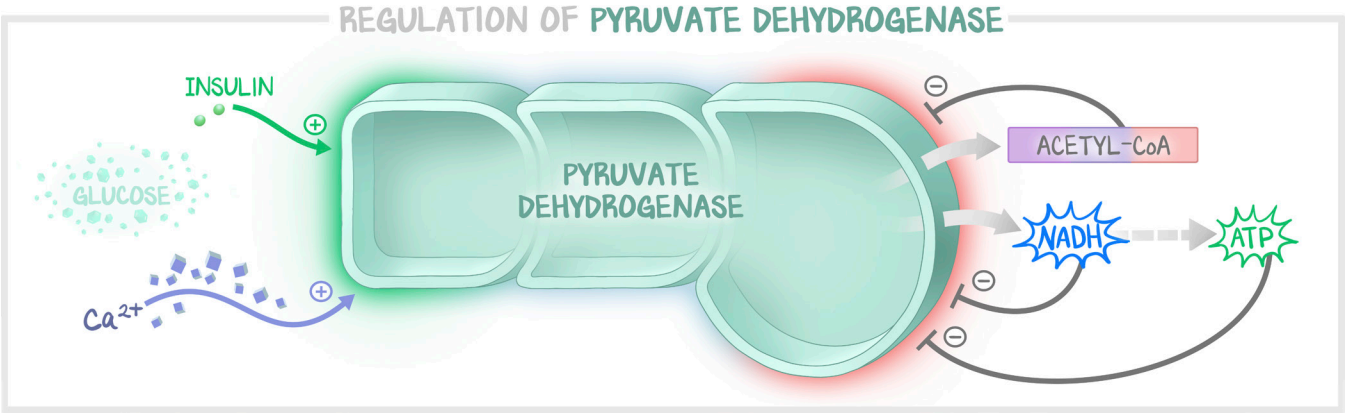

Figure 9.4: Regulation of PDH
Summary from lesson 4, this shows how high-energy compounds feed back to inhibit pyruvate dehydrogenase. In the liver, insulin can override that substrate-level regulation.

The rate-limiting step of the **citric acid cycle** is **isocitrate dehydrogenase**. The product of that enzyme is high-energy. **NADH** inhibits isocitrate dehydrogenase, as does a high-energy state. Both isocitrate dehydrogenase and pyruvate dehydrogenase work similarly, both are inhibited by high-energy states (abundance of NADH, $FADH_2$, ATP) and both are stimulated by low-energy states (abundance of AMP, ADP, NAD, and FAD).

Perhaps the most impressive substrate-level control is **acetyl-CoA**. Acetyl-CoA can't be turned into glucose. β Oxidation of fatty acids results in acetyl-CoA. β Oxidation of fatty acids happens in the starving state, when glucose isn't abundant. The liver is using a different mechanism to generate energy for the rest of the body at the same time it's building glucose. This is impressive because increased levels of acetyl-CoA are a sign that the liver is generating energy for the body, at the exact time it should be doing gluconeogenesis. And yet none of that acetyl-CoA can be turned into glucose. Instead, acetyl-CoA both **inhibits pyruvate dehydrogenase** AND **stimulates pyruvate carboxylase**. The burning of fatty acids, though completely unrelated to carbohydrate metabolism, ensures that the hepatocyte function is aligned—either the liver is burning fatty acids to supply the energy to make glucose OR it is burning glucose in order to store excess ATP as fatty acids. This allows similar substrates (PEP, pyruvate) to be used in both pathways, and the liver "knows" which way to go.

In Perspective

In the insulin-dominant state, ATP comes from glucose (glucose, pyruvate, acetyl-CoA, TCA, ETC). When the cell has enough ATP, the liver is still pushed down glycolysis so that excess acetyl-CoA can be used to make fatty acids. Insulin therefore pushes PFK-1 harder, to make more pyruvate despite the substrate-level regulation fighting it (lots of energy favors FBPase). At the same time, insulin makes PDH work harder, making more acetyl-CoA for FA synthesis, even though substrate-level regulation is inhibiting PDH. When insulin is around, it pushes liver to be like every other cell, only with more functions around glycolysis (FA synthesis) requiring that insulin be around to ensure forward reactions. **When glucagon-dominant, the "extra push" is no longer there**.

In the glucagon-dominant state, ATP comes from FA (FA, acetyl-CoA, TCA, ETC). This ensures that the liver cell has enough ATP, **because** the liver now needs to make glucose for all other cells. FA catabolism replaces glucose as the source of acetyl-CoA and therefore also of NADH, $FADH_2$, and ATP. Now substrate-level regulation does its normal work (there is no "extra push" without insulin), ensuring that **PDH does NOT** make acetyl-CoA, leaving the pyruvate-in-the-mitochondria for

gluconeogenesis. In addition, glucagon ensures that gluconeogenesis progresses by inducing **new enzymes** that no other cell has (PEPCK) and by **undoing insulin regulation** (PFK-2).

How This Fits In

Learn the following:

1. Hormone: Insulin induces glucokinase, dephosphorylates PFK-2, dephosphorylates PDH
2. Hormone: Glucagon induces PEPCK, phosphorylates PFK-2
3. Substrate: NADH, ATP, and acetyl-CoA inhibit pyruvate dehydrogenase
4. Substrate: NADH inhibits isocitrate dehydrogenase
5. Substrate: ATP stimulates FBPase, AMP inhibits it

Then realize that as we continue education on metabolism, we're going to see how that substrate-level control actually works, and why it's so important to command the details. Whenever you encounter regulation of a future system, return to this lesson to see how it fits in.